Designation: D7819 – 12 (Reapproved 2016)

# Standard Test Method for Enumeration of Yeast and Mold on Fresh (Uncured) Hides and Skins[1]

This standard is issued under the fixed designation D7819; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This test method covers the enumeration of yeast and mold on fresh (uncured) hides and skins. This test method is applicable to uncured hides and skins.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

## 2. Referenced Documents

2.1 *ASTM Standards:*[2]

D6715 Practice for Sampling and Preparation of Fresh or Salt-Preserved (Cured) Hides and Skins for Chemical and Physical Tests

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

## 3. Summary of Test Method

3.1 Samples of uncured hides and skins are serially diluted and plated on agar containing an antibiotic solution. The plates are incubated at 20 – 25°C for 5 days.

## 4. Significance and Use

4.1 This test method enumerates yeast and mold. Yeast and mold have been known to cause damage to hides and skins.

## 5. Apparatus

5.1 *Incubator,* 20 – 25°C.

5.2 *Colony counter* (not mandatory, but highly recommended).

5.3 *Sterile pipets.*

5.4 *Stomacher,* for mixing initial dilution. (If stomacher is unavailable, hand-mix.)

5.5 *Balance.*

5.6 *Sterile petri dishes.*

5.7 *Autoclave (sterilizer).* (Check the effectiveness of sterilization weekly. For example, place spore suspensions or strips of *Bacillus stearothermophilus* (commercially available) inside glassware for a full autoclave cycle. Follow manufacturer's directions for sterilization of specific media.)

5.8 *pH meter.*

5.9 *Waterbath,* 45 ± 1°C.

5.10 *Stomacher bags,* or sterile, sealable quart plastic bag (e.g. Food storage type, sterile bag).

5.11 *Cutting tool,* sterile (e.g. scalpel blade and forcep, as needed for cutting cured hides and skins).

5.12 *Vortex mixer,* for mixing dilution tubes (optional).

5.13 *Autoclave thermometer,* or equivalent for monitoring autoclave temperature.

## 6. Reagents and Materials

6.1 *Butterfield's Phosphate Stock Solution:* Dissolve 34 g $KH_2PO_4$ (Potassium Phosphate monobasic) in 500 mL DI water. Adjust the pH to 7.2 ± 0.1 with 1N – 6N NaOH. Bring volume to 1 L with DI water. Sterilize for 15 min at 121°C.

NOTE 1—Typical autoclave setting is 120 – 124°C at 15 psi. (See 5.7.)

6.2 *Butterfield's Phosphate Diluent (BPD):* Take 1.25 mL of Butterfield's Phosphate Stock solution (6.1) and bring to 1 L with DI water. Dispense into 1-litre bottles and 9-mL dilution tubes. Sterilize for 15 min at 121°C. (See Note 1.)

6.3 *Potato Dextrose Agar (PDA).*

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.02 on Wet Blue.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

6.4 *Antibiotic solution* (Chloramphenicol)[3] – (needed to inhibit bacterial growth on agar).

6.5 *Distilled or deionized water.*

6.6 *NaOH, 1N – 6N.*

6.7 *Bacillus stearothermophilus* spore suspensions or strips (commercially available), or equivalent.

## 7. Hazards

7.1 All reagents and chemicals should be handled with care. Before using any chemical, read and follow all safety precautions and instructions on the manufacturer's label or MSDS (Material Safety Data Sheet).

## 8. Sampling

8.1 The specimen shall be sampled in accordance with Practice D6715, and placed in sterile containers.

## 9. Preparation of Potato Dextrose Agar and Antibiotic Solution

9.1 Prepare the antibiotic stock (10 000 ppm) solution by dissolving 1 g of chloramphenicol in 100 mL sterile deionized or distilled water. Store this stock solution in a dark location at ≤5°C for up to two months.

9.2 Suspend 39 g of Potato Dextrose Agar in 1 litre of deionized or distilled water and heat to boiling to dissolve completely.

9.3 Add 10 mL of chloramphenicol stock solution per litre of agar to give a concentration of 100 ppm. Sterilize in the autoclave for 15 min at 121°C. (See Note 1.) Cool to 45 ± 1°C in a waterbath. Once medium has been tempered, it can be held for 2 – 3 h before use, provided the water level in the waterbath is 2 – 3 cm above the surface of the agar. Final pH of the agar: 5.6 ± 0.2.

## 10. Procedure

10.1 Using a sterile scalpel, aseptically obtain a 20 ± 0.1 g specimen that includes both the flesh side and the hair side. Weigh it into a sterile bag. Add 180 g of BPD (6.2) diluent into the same sterile bag. Stomach or hand-massage for 1 min. This provides a 1:10 dilution.

10.2 Prepare the following sample dilutions: $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, and $10^{-7}$ (see Fig. 1).

Example: To obtain a $10^{-2}$ dilution, mix the $10^{-1}$ dilution and pipet 1 mL of that $10^{-1}$ dilution into a 9-mL dilution tube.

NOTE 2—When transferring the aliquots between the tubes, the analyst must use a different pipet or pipet tip for each transfer.

10.3 Pipet 1 mL of each dilution into the appropriate, separate petri dishes.

10.4 Pour prepared agar (9.3) that has been previously tempered to 45 ± 1°C into the dish.

NOTE 3—Add agar within 1 – 2 min after adding dilution to avoid adherence of sample to bottom of dish. Do not pour agar directly on the sample. Replace the cover.

10.5 Swirl the plate gently in a figure-eight motion to evenly distribute the sample.

10.6 Allow agar to solidify.

10.7 Incubate at 20 – 25°C for 5 days (a cabinet at room temperature is acceptable for use).

[3] The sole source of supply known to the committee at this time is Sigma-Aldrich, Cat. # C0378 (25 g). If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

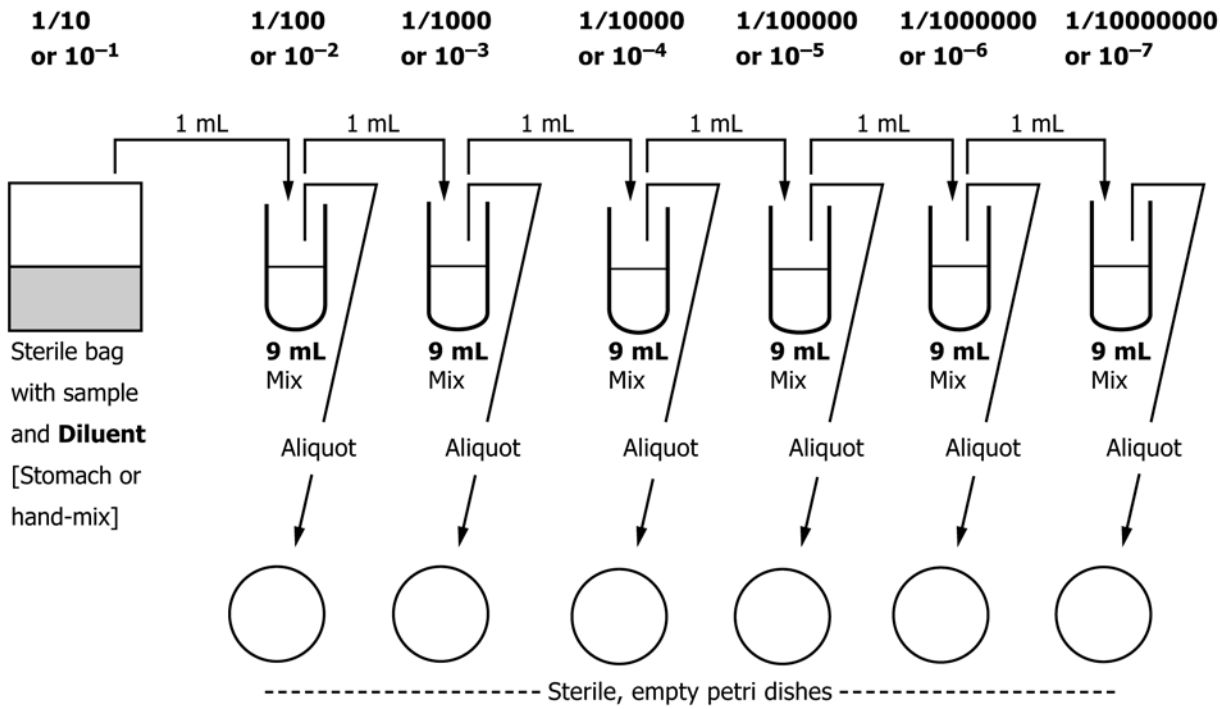


FIG. 1 Plating

NOTE 4—Do not stack plates higher than 3, and do not invert the plates. Let plates remain undisturbed until time for counting. Moving the plates could dislodge spores, thus creating extraneous growths that are not part of the original colony.

10.8 Following incubation, count only those plates that have 10 – 150 colonies.

NOTE 5—Estimated counts can be made on plates with >150 colonies: report as estimated counts. In making such counts, the standard 15 × 100 mm petri dish is considered to have an area of about 56 $cm^2$, therefore, use a factor of 56 when estimating the count. Example: 1 mL of a $10^{-4}$ dilution was plated and the plate has an average count of 5 colonies per $cm^2$. Therefore, the estimated count for that plate is 5 × 56 = 280, and the estimated count for that dilution is 280 × 10,000 = 2,800,000. Estimated counts can also be made on plates with <10 colonies: report as estimated counts.

10.9 Record each plate's dilution and count on the worksheet. Record the yeast count as A, and record the mold as B.

NOTE 6—Yeast colonies will appear as 2 – 3 mm in diameter with a satin-like or matte finish. Most are opaque white, but they may be pigmented (orange or pink), sometimes yellow. Most produce a fermented fruity or bakery aroma. They are convex or conical (raised off the surface) in shape.

NOTE 7—Mold colonies will have a whiskery or cotton tuft-like appearance and may tend to spread over the surface of the agar. They are usually gray, brown, blue-green and green in color, and sometimes become dark gray or even black. Count from the underside of the plate when mold overgrowth has occurred. If mold colonies are present, do not open the plates. Tape them shut before proper disposal.

NOTE 8—If mainly yeasts are present, plates with 150 colonies are usually countable. However, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may be lowered at the discretion of the analyst.

## 11. Calculation of Results for Yeast and Mold

11.1 Calculate by using the following formula:

$$\text{Yeast} = A \times C \quad (1)$$

where:

$A$ = number of colonies counted in step 10.9, and
$C$ = dilution factor (see Table 1).

$$\text{Mold} = B \times C \quad (2)$$

where:

$B$ = number of colonies counted in step 10.9, and
$C$ = dilution factor (see Table 1).

## 12. Report

12.1 Report the results from 11.1 as Yeast per g of *sample* and Mold per g of *sample*, respectively.

NOTE 9—When requested, the counts for yeast and mold may be combined as one count, and reported as "Yeast & Mold".

## 13. Precision and Bias

13.1 The precision of this test method is based on an interlaboratory study of D7819, conducted in 2010. Two laboratories participated in this study. Each of the two laboratories reported seven replicate test results for both the number of colony forming units per gram, and the Log yeast and mold, of a single uncured hide material. Every "test result" reported represents an individual determination. Except for the use of only two laboratories, and a single material, Practice E691 was followed for the design and analysis of the data; the details are given in an ASTM Research Report.[4]

13.1.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "*r*" value for that material; "*r*" is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

13.1.1.1 Repeatability limits are listed in Tables 2 and 3.

13.1.2 *Reproducibility Limit (R)*—Two test results shall be judged not equivalent if they differ by more than the "*R*" value for that material; "*R*" is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

13.1.2.1 Reproducibility limits are listed in Tables 2 and 3 below.

13.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

13.1.4 Any judgment in accordance with statements 13.1.1 and 13.1.2 would normally have an approximate 95 % probability of being correct, however the precision statistics obtained in this ILS must not be treated as exact mathematical quantities which are applicable to all circumstances and uses. The limited number of materials tested and laboratories reporting results guarantees that there will be times when differences greater than predicted by the ILS results will arise, sometimes with considerably greater or smaller frequency than the 95 % probability limit would imply. The repeatability limit and the reproducibility limit should be considered as general guides, and the associated probability of 95 % as only a rough indicator of what can be expected.

13.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

13.3 The precision statement was determined through statistical examination of 28 test results, from two laboratories, on a single uncured hide material.

**TABLE 1 Dilution Factor**

| Test Tube Plated (10.3) | Dilution Factor |
|---|---|
| $10^{-2}$ | 100 |
| $10^{-3}$ | 1,000 |
| $10^{-4}$ | 10,000 |
| $10^{-5}$ | 100,000 |
| $10^{-6}$ | 1,000,000 |
| $10^{-7}$ | 10,000,000 |

[4] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D31-1018.

**TABLE 2 Colony Forming Units per gram**

| | Avg[A] | Repeatability Standard Deviation | Reproducibility Standard Deviation | Repeatability Limit | Reproducibility Limit |
|---|---|---|---|---|---|
| | $\bar{x}$ | $s_r$ | $s_R$ | $r$ | $R$ |
| Fresh (uncured) hide | 68643 | 14807 | 15379 | 41461 | 43062 |

[A] The average of the laboratories' calculated averages.

**TABLE 3 Log Yeast and Mold**

| | Avg[A] | Repeatability Standard Deviation | Reproducibility Standard Deviation | Repeatability Limit | Reproducibility Limit |
|---|---|---|---|---|---|
| | $\bar{x}$ | $s_r$ | $s_R$ | $r$ | $R$ |
| Fresh (uncured) hide | 4.826 | 0.100 | 0.103 | 0.280 | 0.290 |

[A] The average of the laboratories' calculated averages.

## 14. Keywords

14.1 hides; mold; skins; yeast